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Research paper

Complementary methods for contact hypersensitivity (CHS) evaluation in mice

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ABSTRACT

Contact hypersensitivity (CHS) is an experimental model of allergic contact dermatitis (ACD) that can be studied in mice. For CHS responses, mice are immunized by painting with a reactive hapten, such as 1-fluoro-4,6-dinitrobenzene (DNFB), on the shaved abdominal and chest skin. Subsequently, the ears are challenged with diluted hapten, eliciting 'hypersensitive' ear-swelling reactions, which can be measured with a micrometer. In this manuscript we present complementary methods that can be used to evaluate CHS in mice that include: ear weight, vascular permeability, myeloperoxidase (MPO) activity, IFN-γ concentration in ear extracts and also IFN-γ production by auricular lymph node cells (ELNC). The biochemical evaluation of CHS can be also supported by proliferation assay, measurement of IFN-γ production by skin-draining lymph node cells employing ELISA test and by evaluation of IFN-γ^+ TCRαβ CD8 cells with the use of flow cytometry.

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1. Introduction

Allergic contact dermatitis (ACD) is a common skin disease that is caused by type IV delayed-type hypersensitivity responses to antigens that come into contact with the skin (Nosbaum et al., 2009). Contact hypersensitivity (CHS) is an experimental model of ACD that can be studied in mice (Christensen and Haase, 2012). CHS responses can be mediated by either CD4$^+$ Th1, MHC class II-restricted cells locally releasing IFN-г to recruit a characteristic inflammatory infiltrate (Askenase et al., 2004), or by CD8$^+$ MHC class I-restricted Tc1 cells that can similarly release IFN-г, but predominantly mediate cytotoxic damage to local skin cells such as keratinocytes (Kalish and Askenase, 1999; Gocinski and Tigelaar, 1990) and now also IL-17 producing Th17 cells (Zhao et al., 2009; He et al., 2009). More recently it was shown that liver NK cells can play the role of CHS effector cells (O'Leary et al., 2006; Paust et al., 2010).

CHS can be induced by topical skin immunization with reactive chemical haptens dissolved in organic solution. Tc1-mediated CHS induced via skin immunization with DNFB belongs to most frequently studied animal models of skin hypersensitivity (Askenase et al., 2011). Contact hypersensitivity is divided into inductive and effector limbs. The inductive limb results from hapten immunization and takes about 4–5 days. After painting of the chest and the abdominal skin with the hapten, it penetrates through the stratum corneum barrier of the skin. During this step the sensitizer binds to the self proteins in the skin (Kaplan et al., 2012). Then, hapten-conjugated self proteins are recognized

Abbreviations: ACD, allergic contact dermatitis; CHS, contact hypersensitivity; DDC, dermal dendritic cells; DNFB, 1-fluoro-4,6-dinitrobenzene; DNP, 2,4-dinitrophenol; DNP-BSA, 2,4-dintrophenylated bovine serum albumin; ELNC, auricular lymph node cells; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; IFN-γ, interferon-gamma; IL-1β, interleukin 1-beta; IL-17, interleukin 17; LNC, lymph node cells; MHC, major histocompatibility complex; MPO, myeloperoxidase; NK, natural killer cells; NKT, natural killer T lymphocytes; OD, optical density; SPF, specific pathogen free; Tc1, type 1 cytotoxic lymphocytes; Th1, type 1 T helper lymphocyte

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by innate immune mechanisms in the skin that results in the induction of pro-inflammatory cytokines such as IL-1β (Suttervala et al., 2006; Martin et al., 2011). This leads to the activation of dermal dendritic cells (DDC). The activated DDC that are directly haptenated or picked up hapten-conjugated self proteins migrate to the draining lymph nodes, where they induce CHS-effector T cells which are ready to play their effector function within 4–5 days post sensitization (Kaplan et al., 2012). Additionally, within 1 day post hapten immunization liver NKT and peritoneal B-1 B cells are activated and then participate in the recruitment of CHS-effector T cells (Askenase et al., 2011; Kerfoot et al., 2008). The role of NKT and IL-4 and B cells in CHS initiation was confirmed by experiments employing CD1d −/−, Jα18 −/−, IL-4Rα −/−, STAT6 −/− and pan-B cell deficient JH −/− mice respectively (Askenase et al., 2011).

The effector limb is elicited, following secondary challenge with the same hapten. It was previously shown that effector limb of CHS has biphasic nature and is divided into two phases (Askenase et al., 2004). First, an elicited initiating phase very early after hapten challenge, and, second, a classical late inflammatory phase mediated by the recruited CHS-effector T cells. The elicited early component peaks at 2 h following the hapten challenge and is due to an "initiation process" that results in local recruitment of antigen-specific effector T cells to the site of elicitation e.g. the ears to begin the late phase (Askenase et al., 2004). The CHS-effector T cells release pro-inflammatory cytokines such as IFN-γ and start killing the hapten-labeled cells e.g. keratinocytes which results in edema — ear swelling that can be measured with a micrometer 24 h after the challenge (Askenase et al., 2004).

The aim of this paper is to present objective laboratory methods that may help to confirm ear swelling measurements with a micrometer.

2. Materials and methods

2.1. Mice

Specific pathogen free (SPF) male BALB/c (H-2^d) mice were from the breeding unit of the Department of Medical Biology, Jagiellonian University College of Medicine. Mice were rested for at least 1 week before use, maintained under specific SPF and used at 6–10 weeks of age in groups of 5–6. All experiments were conducted according to guidelines of Jagiellonian University College of Medicine. All experiments were repeated at least three times, and a representative experiment is shown in the figures.

2.2. Reagents

1-fluoro-4,6-dinitrobenzene (DNFB), Evans blue, formamide, hexadecyltrimethyl-ammonium bromide and o-dianisidine dihydrochloride were obtained from Sigma, St. Louis, MO. IFN-γ BD OptEIA Set was from BD Biosciences, San Diego, CA. 2,4-dintrophenylated bovine serum albumin (DNP-BSA) was from Biosearch Technologies, Inc., Novato, CA.

2.3. Immunization and elicitation of CHS

Unanesthetized mice were contact sensitized with 25 μl of 0.5% DNFB in acetone: olive oil (4:1) applied by painting on the shaved abdominal and chest skin (positive control) or sham immunized by painting with 25 μl of vehicle alone (negative control). Five days later, CHS responses were elicited by painting both ears with 5 μl of 0.1% DNFB in acetone and olive oil (1:1) (Askenase et al., 2011; Kerfoot et al., 2008). Resulting ear thickness was measured prior to testing with a micrometer (Mitutoyo, Tokyo, Japan) by an observer unaware of the experimental groups and then again at 24 h after challenge. Increased ear thickness was expressed as the mean ± SE.

2.4. Measurement of ear weight

To determine ear weight animals were sacrificed 24 h after challenge. The ears were removed and 6 mm diameter punch in central part of each ear was made with biopsy punch (Frey Products Corp., cat# BP60). The weight of the punches was measured with the use of analytical laboratory scale (Sartorius).

2.5. Vascular permeability test

To assess very early changes in vascular permeability, DNFB immune or naïve mice were challenged with 5 μl of 0.1% DNFB and injected with 1% Evans blue dye (83 μg/g body weight) 23 h later. 1 h after Evans blue injection mice were anesthetized and sacrificed. Ears were removed and 6 mm diameter punch in central part of the ear was made with biopsy punch (Frey Products Corp., cat# BP60). Ear punches were transferred to tubes containing 1 ml of formamide. After 18 h incubation at 37 °C the samples were centrifuged and the optical density (OD) of the supernatant fluids was read at 565 nm against blank containing formamide alone (Majewska-Szczepanik et al., 2012).

2.6. In vitro measurement of IFN-γ in CHS ear extracts

To determine local production of IFN-γ in elicited DNFB CHS, mice were immunized with 0.5% DNFB or sham sensitized and 5 days later challenged with 5 μl of 0.1% DNFB. Ears were removed 24 h post challenge and 6 mm diameter punch in central part of the ear was made. The biopsies were collected from the distal site of CHS ear responses and were frozen rapidly in liquid N_2 and then were subsequently thawed and extracted in 300 μl cold PBS on ice with a tissue micro-homogenizer. Concentration of IFN-γ was measured by ELISA with the use of BD OptEIA Set (BD Biosciences, San Diego, CA) (Majewska-Szczepanik et al., 2012).

2.7. Myeloperoxidase (MPO) assay

Neutrophil infiltration to the inflamed ears was indirectly quantitated using an MPO assay, as described previously (Szczepanik et al., 2000). Ears were removed 24 h post challenge and 6 mm diameter punch in central part of the ear was made. The biopsies were collected from the distal site of CHS ear responses and were homogenized in 0.5% hexadecyltrimethylammonium bromide pH = 6.0 (50 mg of tissue/ml). The homogenates were freeze–thawed 3 times, centrifuged at 40,000 *g* and then 20 μl aliquots were mixed with 200 μl phosphate buffer (pH = 6.0) containing 0.167 mg/ml o-dianisidine dihydrochloride and 5×10^{-4} % H_2O_2 and

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